TESTING FOR EPINEPHRIN (ADRENALIN) IN BLOOD. COMPARISON OF PLASMA AND SERUM.

BY G. N. STEWART, M.D.

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TESTING FOR EPINEPHRIN (ADRENALIN) IN BLOOD. COMPARISON OF PLASMA AND SERUM.*

BY G. N. STEWART, M.D.

(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland.)

Certain observers have stated that the pressor effect of defibrinated blood or serum is due to substances produced in clotting. If this be so, it is obvious that the complication introduced by this property in testing for epinephrin in blood, which was discussed in a previous paper,[1] can be avoided by preventing the blood from coagulating by the addition of some substance which does not itself interfere with the epinephrin reactions. I have put the question to the test as regards the two objects, intestine and uterus, employed in the previous work, by comparing the action of blood or plasma prevented from clotting by hirudin with the action of the same blood after defibrination or of the serum separated from it. Both dog blood and human blood were used and the result was the same in both cases; that is, that the tone-increasing power of the unclotted blood or plasma for segments of rabbit intestine and uterus was practically identical with that of the defibrinated blood or serum. Possibly in some instances the increase of tone in the intestine produced by the plasma or by the uncoagulated blood was somewhat more transient than that produced by the serum or defibrinated blood. But this is by no means constant and in any case the difference was quite inconspicuous. Serum separated spontaneously from the clot had, as was to be expected, a precisely similar action to that of serum separated by the centrifuge from the defibrinated blood.

It follows from these results that, at least so far as heterologous blood is concerned, no advantage is to be gained by using hirudin blood or plasma instead of defibrinated blood or serum in testing

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[1] Stewart, G. N., *Jour. Exper. Med.*, 1912, xv, 547.

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for epinephrin by the rabbit intestine and uterus reactions. A further consequence of these observations is that the substance or substances responsible for the tone-increasing power of serum on intestine and uterus segments initially beating in Ringer's solution are not produced in the process of coagulation but preëxist in the unclotted blood. In proof of these statements the protocols of two experiments, one with dog and the other with human blood, are cited, and specimens of the tracings reproduced.

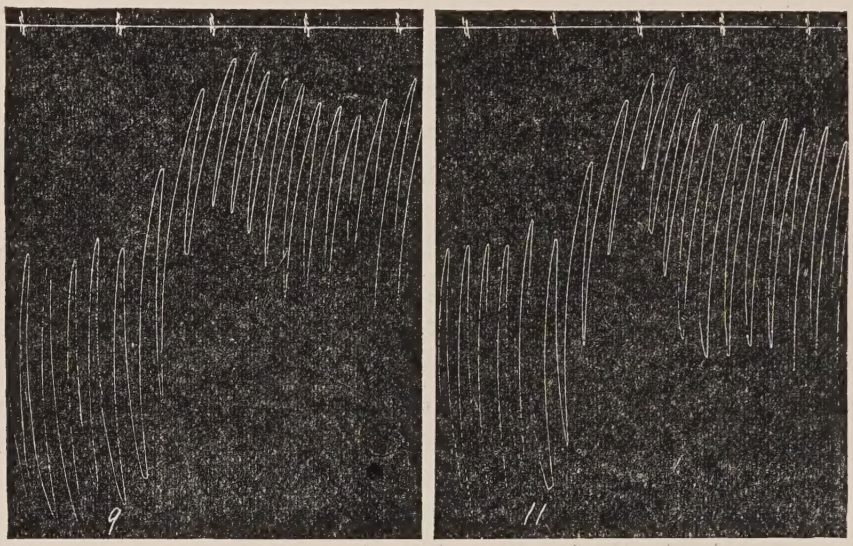
Experiment I.—A solution of hirudin in Ringer's solution containing 0.01 gm. in 1 c.c. was made. The hirudin, just obtained from the importer, was sealed in a glass tube, which was broken to make the solution.

At 11 A. M. 25 c.c. of blood from the carotid artery of a small bitch (under ether anesthesia) was run into 5 c.c. of the hirudin solution. The animal had a large goiter and the flow from the enlarged carotid was very rapid. Some blood was first allowed to escape from the cannula before collection was begun, in order to avoid all risk of the presence of clot. The blood was mixed well with the hirudin solution. No clotting occurred, even in several days. Immediately afterwards, another specimen of blood was collected and defibrinated. Ringer's solution to the amount of one fifth of the volume of the defibrinated blood was added to it so that it should be diluted to the same extent as the hirudin blood. Then the rest of the blood was at once drawn from the animal and allowed to clot after the addition of one fifth of its volume of Ringer's solution. Serum was separated immediately by the centrifuge from the defibrinated blood, and plasma from the hirudin blood. The yield was large and in both cases the liquid was free from blood pigment. The same was true of the serum from the clot, which separated very quickly.

At 2.25 P. M. tests were begun with an intestinal segment of a rabbit. Specimens of the curves are given in text-figures 1 to 3. The smaller apparatus was used, 2 c.c. of liquid being always run into it. The weight of the intestine preparation was 0.46 gm.

At observation 9 (text-figure 1) Ringer's solution in which the preparation was beating was replaced by serum from the defibrinated blood. After washing, the preparation was again beating in Ringer's solution when, at 11, this was replaced by plasma from the hirudin blood. It will be seen that the maximum increase of tone produced by the plasma was almost the same as that produced by the serum. At 13 (text-figure 2) hirudin blood replaced the Ringer and caused a considerable increase of tone. After washing, the preparation was again beating in Ringer's solution when this was replaced, at 15, by the defibrinated blood. The initial tone of the preparation before the addition of the defibrinated blood was less than its initial tone

before the addition of the hirudin blood. (The exact relations of the two curves to the base line are preserved in the figure and no change in position of the lever was made between the two observations.) It might, therefore, be expected that if the tone-increasing power of the two specimens was really equal, the absolute increase



All the tracings are to be read from left to right. Time is marked in half minutes.

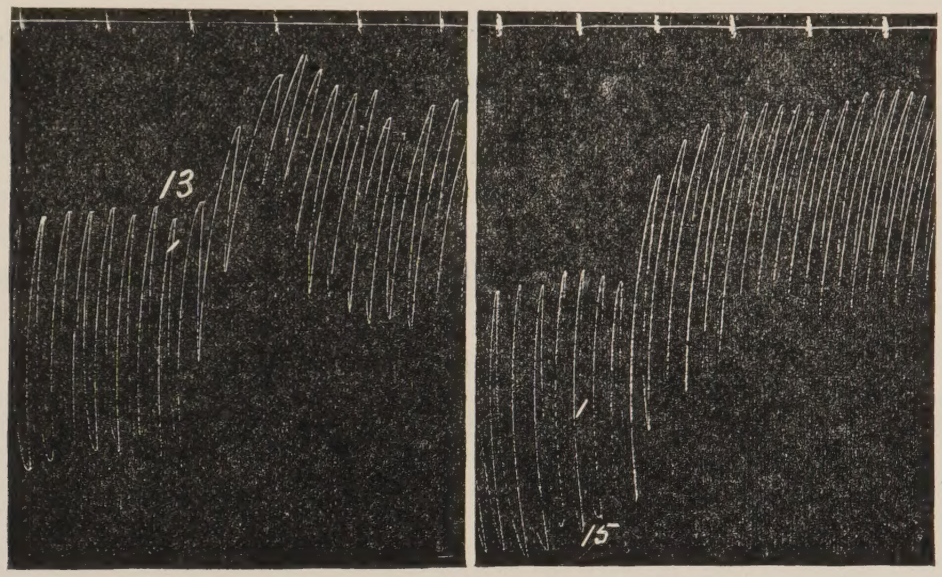
Text-figures 1 to 3 were obtained from the same rabbit intestine preparation; text-figures 4 to 6 from an intestine preparation from another rabbit. The magnification of the contractions by the lever was somewhat more than twice as great in text-figures 4 to 6 as in 1 to 3. The smaller apparatus holding 2.5 c.c. without the preparation was used throughout. 2 c.c. of the liquid to be tested, *i. e.*, enough to replace all of the liquid initially present, were always added. At the beginning of each observation the preparations were beating in Ringer's solution, the serum, etc., if previously employed, having been thoroughly washed away with Ringer.

TEXT-FIG. 1. At 9 serum from defibrinated dog blood replaced Ringer. At 11 plasma from hirudin dog blood replaced Ringer.

would be greater with the defibrinated blood, as is seen to be the case. On the other hand, the maximum height is greater for the hirudin blood. In other observations it was shown that where the initial tone of the preparation is the same, the absolute increase of tone produced by hirudin plasma is practically the same as that produced by serum from defibrinated blood.

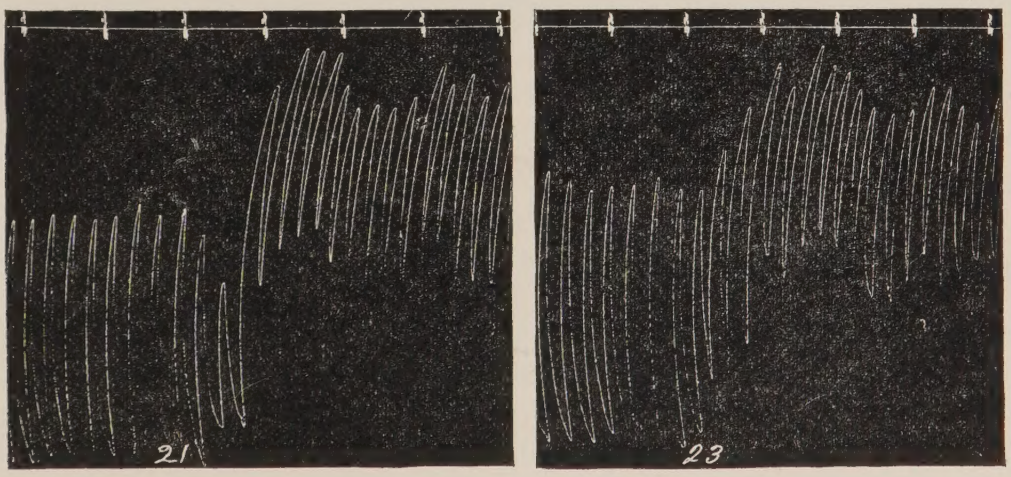
In text-figure 3 at 21 serum from the clot replaced Ringer's solution. After washing the preparation the Ringer's solution was

replaced, at 23, by hirudin plasma. The initial tone of the preparation before 23 was somewhat greater than before 21, and the same remark applies to the comparison that has been made in comparing



TEXT-FIG. 2. At 13 hirudin dog blood replaced Ringer; at 15 defibrinated dog blood replaced Ringer.

observations 13 and 15. The magnification of the contractions in figures 1 to 3 is somewhat less than half the magnification in text-



TEXT-FIG. 3. At 21 serum from clotted dog blood, at 23 plasma from hirudin dog blood replaced Ringer.

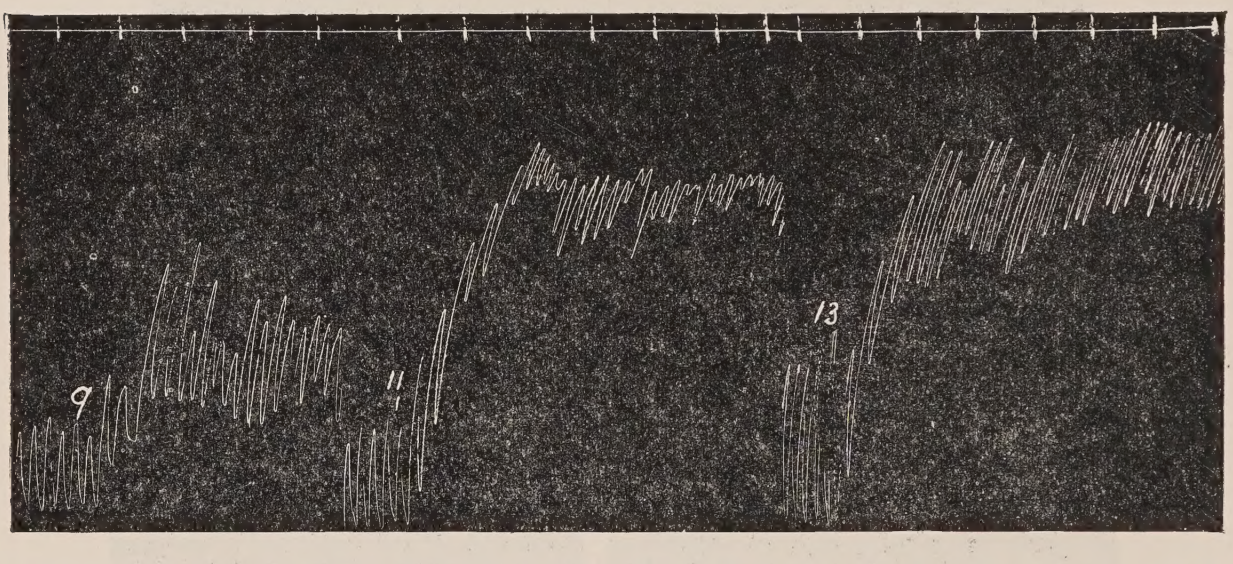
figures 4 to 6. The higher magnification was used in most of the experiments in the previous paper. This is pointed out lest the

effects in text-figures 1 to 3 might be underestimated by a reader who looks casually at the tracings.

The uterus tests showed no constant difference between the various specimens.

Experiment II.—Blood was obtained by puncture of an arm vein from F. W., a man aged 44 years, with mitral insufficiency and a history of lues several years ago.[2]

Just before the vein was tapped, 0.04 gm. of hirudin was dissolved in 4 c.c. of Ringer's solution, and 22 c.c. of blood were then run into the solution. The test-tube was agitated constantly as the blood ran in so as to insure thorough mixture of the blood and hirudin solution. The flow of blood was free. The first portion coming through the cannula was rejected. No clotting whatever



TEXT-FIG. 4. At 9 serum from F. W.'s clotted blood, at 11 his hirudin plasma, at 13 serum from his clot, replaced Ringer.

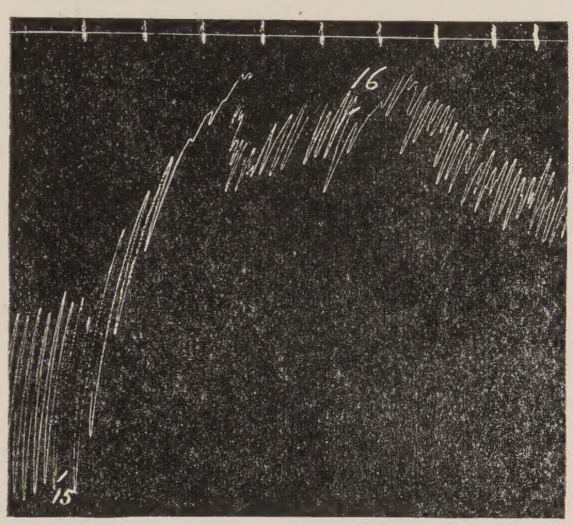
occurred, even in several days. As soon as the hirudin specimen had been obtained, 70 c.c. of blood were at once collected from the same man in another vessel, and allowed to clot. 13 c.c. of Ringer's solution were added to it. Both specimens were immediately conveyed to the laboratory and put in the ice chest over night. Abundance of hemoglobin-free serum and plasma separated.

At 11 A. M. the following morning tests were begun with an intestine preparation from a rabbit. The preparation weighed 0.44 gm. The smaller apparatus was used.

Observation 9 (text-figure 4) shows the effect of replacing Ringer's solution by serum from the clot, and 11 the effect of re-

[2] I am much indebted to Dr. Bucher, of the City Hospital, for aiding me in collecting this material. While Dr. Bucher attended to the tapping of the vein, I gave undivided attention to the proper collection of the hirudin blood.

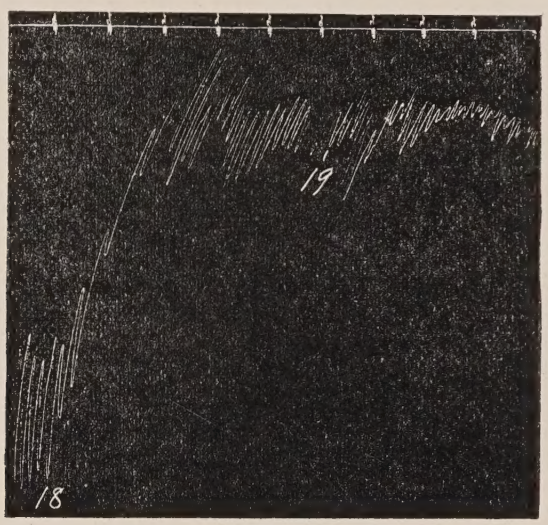
placing Ringer's solution by hirudin plasma. On the face of these two observations alone, the hirudin plasma is so far from being inferior in tone-increasing power that the increase is quite twice as great as that caused by the serum. This, however, is not a fair comparison. For 9 was the first observation in which this intestinal segment had come into contact with serum, and it is sometimes seen that the effect of the first serum bath, particularly in the case of a segment which is not beating very strongly, is to increase the power of response of the preparation to subsequent immersions in serum. This part of the tracing is reproduced here merely to illustrate the



TEXT-FIG. 5. At 15 Ringer was replaced by F. W.'s hirudin plasma, and this at 16 by serum from his clotted blood.

point that repeated observations are essential if errors of interpretation are to be avoided in this kind of work. At 13, after washing the preparation, the Ringer's solution was replaced by serum from the clot. The increase of tone is perhaps slightly greater than in observation 11, but the difference is no more than may be found in successive observations with the same serum. At 15 (text-figure 5) Ringer's solution was replaced by hirudin plasma, which at 16 was replaced by serum from the clot. In text-figure 6 this experiment was performed in the reverse order, serum from the clot replacing Ringer's solution at 18 and being itself replaced by hirudin plasma at 19. If the tone-increasing power of the serum had been conspicuously greater than that of the plasma, the rise at 16 ought to

have been decided and sustained, whereas at 19 there should have been a marked fall. On the contrary, there is a slight rise at 19 just as at 16, a rise of the same order of magnitude as is caused by simple renewal of one and the same medium, and the increase of tone after 19 is actually better sustained than after 16.



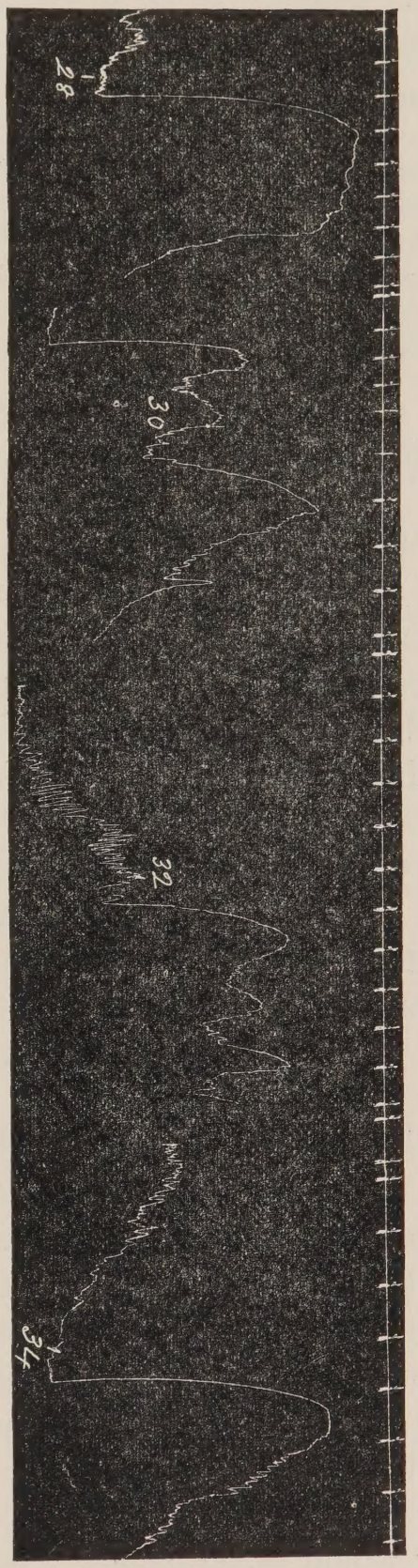
TEXT-FIG. 6. At 18 Ringer was replaced by serum from F. W.'s clotted blood, and this at 19 by his hirudin plasma.

Text-figure 7 illustrates the uterus tests with the same specimens of hirudin plasma and serum from F. W. The uterus preparation (a segment of a horn) weighed 0.58 of a gram. The rabbit was a young adult, not pregnant. The smaller apparatus was used.

At 28 hirudin plasma replaced Ringer's solution. A great increase of tone ensued. After washing, the preparation was again beating in Ringer's solution when, at 30, serum from the clot was run in. Starting from a considerably higher level, the absolute increase of tone is not so great as that produced by the plasma at 28, but is nevertheless quite distinct. At 32 hirudin plasma replaced Ringer's solution at a point corresponding to a somewhat greater tonic contraction of the segment than before observation 28. The rise, while considerable, is less than in 28. At 34, after washing, the tone of the segment was less than in any of the previous observations and the addition of serum was followed by a rise of about the same absolute amount as that caused by the plasma in observation 28.

Certainly in those observations there is nothing to support the idea that substances capable of increasing the tone of the uterus are produced in blood during coagulation. If anything, the plasma caused a greater and more persistent increase of tone than the serum. The difference, however, lay within the limits of variation in such observations even with one and the same liquid.

I have no experience of the perfusion method introduced by Läwen and developed by Trendelenberg, and I am, therefore, not in a position to discuss O'Connor's[3] statement that the pressor effect on perfused frog blood-vessels is almost entirely absent if the blood has been prevented from clotting. He says that the same is true of the tone-increasing power on the rabbit intestine and uterus. He worked, however, in the case of the intestine and uterus with homologous blood. From the point of view of this paper, only the action of heterologous serum and plasma is of interest, since in clinical testing the blood must always be heterologous to the test object. My experiments, therefore, do not strictly compare



Text-Fig. 7. Uterus preparation. At 28 Ringer was replaced by F. W.'s hirudin plasma, at 30 by serum from his clot, at 32 by his hirudin plasma, at 34 by serum from his clot. After each observation the preparation was thoroughly washed with Ringer.

[3] O'Connor, J. M., *Arch. f. exper. Path. u. Pharmakol.*, 1912, lxvii, 195.

with O'Connor's. It is, nevertheless, noteworthy that he makes the following statement:

"Es ist aber keineswegs leicht, in allen Fällen sicher reines Plasma zu gewinnen und die Resultate fallen nicht eindeutig aus, wenn bei der Gewinnung des Plasmas nicht jeder Zellzerfall vermieden wird; aus diesen Grunden ist es auch schwierig, die Unwirksamkeit des Hirudinplasmas auf Darm und Uterus zu erweisen; bei dem Zusammenbringen des Plasmas mit den in Ringerlösung suspendierten Organen entsteht durch die Anwesenheit der Thrombokinasen in den Geweben leicht Gerinnung. Dennoch ist es mir gelungen, sowohl am Darm als am Uterus einwandfreie Versuche zu erhalten."

I can only say that in my experiments every care was taken to collect the hirudin blood under the best conditions. The large dose of hirudin was purposely chosen to insure the absence of the slightest trace of clotting; also, as already mentioned, the flow of blood was very free. Not the slightest indication of clotting was seen at any stage of the experiments. Plasma which had once come into contact with the intestine or uterus preparation was never used a second time. The almost exact quantitative agreement in the action of the plasma and serum appears to me to be a decisive argument against the possibility that the effect of the plasma was due to a certain amount of "cell destruction" which had not been avoided. Had the plasma exerted an effect always conspicuously smaller than that of the serum such a possibility might have been entertained, but it is difficult to see how the effect could be the same unless it is independent of clotting. That occasional instances may occur in which hirudin blood or plasma apparently causes a relatively slight increase of tone while defibrinated blood or serum causes a large increase may be granted. But the converse observation may also sometimes be made, and in interpreting such results the fact that the intestine or uterus preparation varies from time to time in its power of response to one and the same liquid must not be forgotten. For instance, as already pointed out, in text-figure 4 the first two observations (9 with serum, and 11 with plasma), if standing by themselves, would indicate that the tone-increasing power of the serum was much less than that of the plasma, a conclusion which certainly would be erroneous. Suppose that one had happened at 9 to add plasma instead of serum, there is scarcely any doubt that practically the same curves as were actually written would have

been obtained. Had the experiment stopped here the interpretation might have been put upon it that hirudin plasma has a far smaller tone-increasing power than serum, and this conclusion would also have been unjustified. Observation 9, as was shown by subsequent tracings, is an anomaly in the series and must not be used in the comparison. If it were used, it would permit precisely the opposite conclusion to that which O'Connor deduced from his observations. These remarks are far from being intended as a general criticism of O'Connor's interesting paper. His conclusion that epinephrin cannot be demonstrated with certainty in the peripheral venous and arterial blood agrees entirely with that drawn in my previous papers.[4] Even if it be admitted, as O'Connor says it must be, that it is difficult to avoid errors of technique in obtaining plasma suitable for use in the intestine and uterus reactions, this alone is sufficient to show that in epinephrin-testing for clinical purposes by means of these reactions no advantage can be gained by endeavoring to substitute plasma for serum.

[4] Stewart, G. N., *Jour. Exper. Med.*, 1911, xiv, 377; 1912, xv, 547.

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